Mixed biofilm of *Aspergillus fumigatus* and *Stenotrophomonas maltophilia*: a strategy of protection against antimicrobial agents?

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The mold *Aspergillus fumigatus*, and the gram-negative bacillus *Stenotrophomonas maltophilia* can form biofilms, especially in lungs and airways of immunocompromised hosts and cystic fibrosis patients. Recently, we have developed an *in vitro* mixed biofilm model associating these opportunistic pathogens. In biofilms, microorganisms are embedded in an extracellular matrix (ECM), which may confers protection against antimicrobial agents.

The aim of this study was to test *in vitro* two antifungal and two antibacterial agents on single and mixed biofilms associating *A. fumigatus* (ATCC 13073-GFP), and *S. maltophilia* (ATCC 13637).

Does fungal-bacterial biofilm provide a protective lifestyle for microorganisms against antimicrobial agents?

Methods

Antifungals = **amphotericin B** or **itraconazole** [256-0,25 µg/ml]

Antibacterials = **levofloxacin** or **rifampicin** [32-0,03 µg/ml]

Antimicrobial susceptibility testing with:

- ∞ **XTT assay**: Evaluation of single biofilm metabolic activity
- ∞ **Crystal violet (CV)**: Measurement of single biofilm biomass
- ∞ **Quantification of colony-forming units (CFUs)**: Cell viability
- ∞ **qPCR**: Quantification of *A. fumigatus* and *S. maltophilia* DNA in single and mixed biofilms

Results

Antifungal susceptibility

Fig 1: *A. fumigatus* susceptibility after exposure to increasing concentrations of antifungal agents. (A) Measure of absorbance at 450 nm (XTT method) in fungal biofilms. (B) Fungal DNA quantification in single and mixed. Inhibition percentage of fungal biomass in treated biofilm compared to untreated biofilm (red point).

1. Amphotericin B can inhibit *A. fumigatus* in single and mixed biofilms with concentration ≤ 256 µg/ml:

- Af is not metabolically active after treatment with 256 µg/ml (fungal biofilm)
- 95% of growth inhibition with 32 µg/ml (fungal and mixed biofilms)

2. Itraconazole have no effect on *A. fumigatus* in single and mixed biofilms with concentration up to 256 µg/ml

Antibacterial susceptibility

Fig 2: *S. maltophilia* susceptibility after exposure to increasing concentrations of antibacterial agents. (A) Metabolic activity (XTT), biomass (CV) and cell viability (CFU) in single bacterial biofilms treated with levofloxacin (A1) and rifampicin (A2). (B) Bacterial DNA quantification in single and mixed biofilms. Inhibition percentage of bacterial biomass in treated biofilm compared to non-treated biofilm (red point).

Levofloxacin and rifampicin have an inhibitory effect on *S. maltophilia* growth and viability in single biofilms but not in mixed biofilms:

- metabolism/biomass of Sm were affected after treatment with concentration <1 µg/ml (single biofilm)
- viability of Sm is reduced by 50% with 2 µg/ml of levofloxacin and 4 µg/ml of rifampicin with CFU assay
- inhibition of growth of 95% with 8 µg/ml (single biofilm)

S. maltophilia is less inhibited by antibacterials in mixed biofilm (20-25%) than bacterial biofilm (93-95%)
>> fungi and/or ECM play a role in this 'protective effect' ?

These results suggest that *A. fumigatus* biofilm may protect *S. maltophilia* from antibacterial agents in mixed biofilm. To complete our study, microscopic analyses will be benefit to assess the viability of microorganisms in mixed biofilm and to examine the drugs diffusion through the ECM. Future experiments to investigate the role of fungal extracellular matrix in bacterial protection can provide ways of explaining the reduced susceptibility of *S. maltophilia* in mixed biofilm.

Conclusions & Future directions